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Validation of a Field Monitoring System for BPU-Type IGR Resistance in the Diamondback Moth, *Plutella xylostella*- Presumed Working Scheme Based on IRAC Method No. 7 (Leaf Dipping Method)[1]

Edward Y. Cheng and Ching-Hua Kao[2]

Abstract：Results of the IRAC Method NO. 7 (leaf dipping method) were compared to those of the spray tower method in aspect of the resistance of diamondback moth (DBM), *Plutella xylostella*, to benzoylphenyl urea (BPU)-type insect growth regulators (IGR). The leaf dipping method resulted in 3.29-fold higher BPU-IGR deposit than the spray tower method. The treating period of leaf dipping lasts from 3rd instar to pupation, which is longer than the 48-hour period in the spray tower method. Both the higher dosage and the longer treating period of leaf dipping had resulted in higher sensitivity of the DBM to the treated doses of BPU-type IGR compared to the spray tower method. No anti-feeding effect of BPU-type IGR against DBM was observed.

The susceptibility of a susceptible strain to 4 BPUs, chlorfluazuron, teflubenzuron, hexaflumuron and flufenoxuron, had been determined by using the leaf dipping method. The reproducibility of this method was tested and substantiated by comparing LC_{50}s in the n and n+6 generations of the susceptible strain. The ability of leaf dipping method in detecting the BPU-resistance in DBM was verified by the Independent Student's t-distribution test, which confirmed the separation of LC_{50}s of the susceptible strain from 3 BPU-resistant strains at 95 % fiducial limits.

We concluded that four to five IGR concentrations in 10-fold increments with water are sufficient for testing BPU-resistance in DBM, except for hexaflumuron, the increment should be adjusted to 4-5 fold. The leaf dipping method is not suitable for synergistic with piperonyl butoxide (PB) due to different methods were applied to the DBM.

Key words：*Plutella xylostella*, BPU-type insect growth regulators, Resistance, IRAC Method No. 7, Leaf dipping method.

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INTRODUCTION

Insecticide resistance has become a serious problem in the IPM program. Many investigators and organizations have proposed resistance management in the application of insecticides. In dealing with this problem, IRAC(Insecticide Resistance Action Committee) in fulfilling its aim of providing expert advice to GIFAP has suggested nine testing methods on the susceptibilities of the insecticide/acaricide[8]. Amongst them, the IRAC Method No. 7 (leaf dipping method) proposed for leaf-eating larvae of Lepidoptera species, particular for cotton, vegetable and field crops, is suitable for testing organophosphates, carbamates, pyrethroids, organochlorines and insect growth regulators (IGRs). The spray tower method is mimic to the real field spray and has been frequently used for studying the insecticide resistance, but the leaf dipping method is more convenient to work with in resistance study, particularly on stomach poison insecticides. We would like to validate the leaf dipping method for BPU-type IGRs on Taiwan's resistant diamondback moth (DBM), *Plutella xylostella* L.

MATERIALS AND METHODS

Insecticides used:

I. For both the dipping and the spraying tests:

Chlorfluazuron (CFA), 5 % EC (Ishihara Sangyo Kaisha Limited), Teflubenzuron (TFB), 15 % SC (Celamerck GmbH & Co. KG), Hexaflumuron (HXF), 10 % EC (DowElanco) , Flufenoxuron (FFO), 10 % EC (Shell International Chemical Company Limited).

II. For the HPLC residue analysis:

CFA, 99.3 % standard (Ishihara Sangyo Kaisha Limited), TFB,98.4 % standard (Celamerck GmbH & Co, KG), HXF, 99.6 % standard (DowElanco), FFO, 97.6 % standard (Shell International Chemical Company Limited).

Synergist and other chemicals :

Piperonyl butoxide (PB) 50 % EC, was formulated by mixing 50ml PB with 5 ml Paracol-L (emulsifier), 5 ml Paracol-MBK (emulsifier) and 40 ml xylene.

Acetonitrile (Uvasol grade) used for HPLC analysis was purchased from Merck. Triton X-100 used for leaf dipping solution was purchased from Sigma Chemical Company.

Insects: Four strains of DBM were used for the test, and they were:

I. Susceptible strain-Taiwan native I-lan (IL) strain.

II. Resistant strains:

i. RS1-with previous TFB usage record.

ii. RS2-with previous TFB and CFA resistance record.

iii. RS3-from CFA selection field.

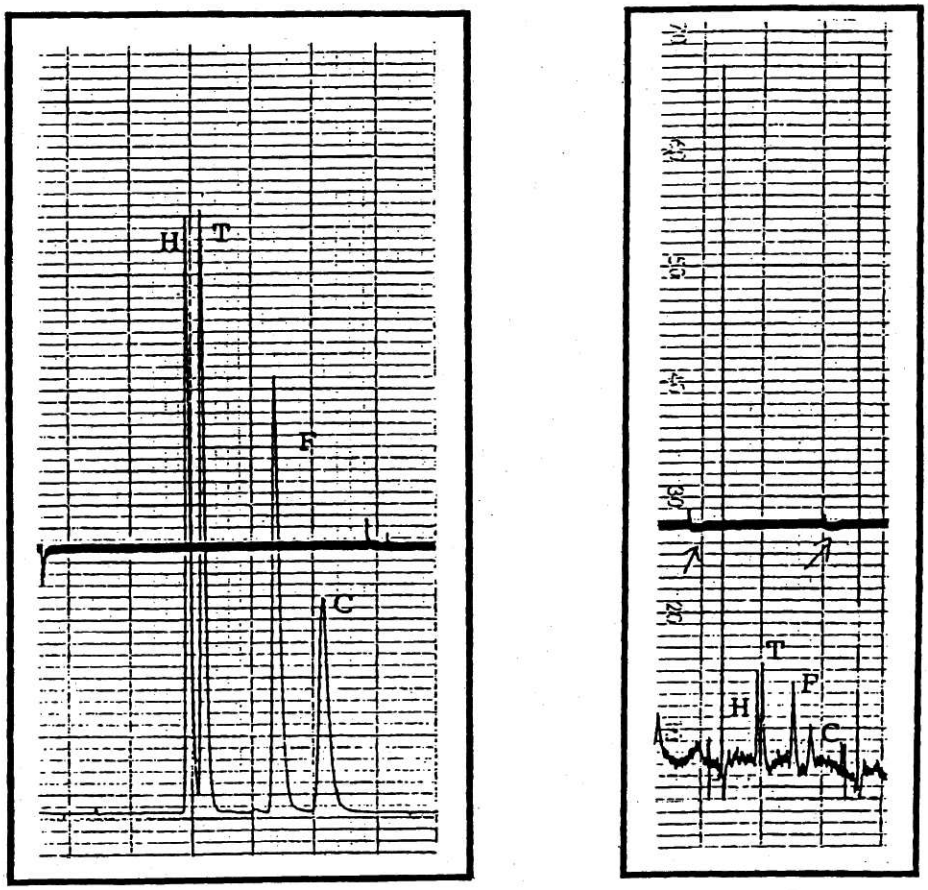
Apparatus and instruments :

High performance liquid chromatography (Gilson) and Burkard Potter Spray Tower were used for the test. Other apparatus used were as described in IRAC Method No.7[8].

BPU residue analysis :

Celamerck Document No. 13406-522-02[1] was adopted for all 4 BPUs residue analysis, and the HPLC analytic conditions were as follows: (1) column:Merck RP-18, 25cm, (2) mobile phase: acetonitrile/water= 65/35, (3) flow rate:2ml/min, (4) pressure: 1,500 psi, and (5) retention times: 3.9, 4.4, 6.7 and 8.1 minutes for HXF, TFB, FFO and CFA, respectively (Fig.1).

Analysis conditions:
Column: RP-18 Merck, 25cm
Mobile phase: Acetonitrile/water(65/35)
Flow rate: 2 ml/min
Pressure: 1,500psi



(a) Chart speed=5 mm/min (b) Chart speed= 2 mm/min

Fig. 1. HPLC determination of 4 BPUs deposition on the cabbage leaf discs
(a) Separation of hexaflumuron (H), teflubenzuron (T), flufenoxuron (F) and chlorfluazuron (C) (Retention times: 3.9, 4.4, 6.7 and 8.1 min)
(b) Determining the detection limit at 5 ng-per BPU

Bioassay methods :

I. Spray Tower method[2,3]: 1 ml of insecticide solution was pipetted into the holding tube of Burkard Potter Spray Tower and sprayed on leaf disc. After air-dried, the leaf disc was inverted and loaded with fifteen 3rd instar DBM larvae, and sprayed with another milliliter of the same insecticide solution. Treated insects and leaf disc were transferred into a petri dish and covered with a half-folded tissue paper before putting on lid. Total of 60 larvae in 4 subsets were treated for each dosage. The post-treatment holding condition was controlled at 25℃ in a constant temperature growth chamber. The leaf disc used was 24 cm^2 and changed to a fresh untreated leaf disc at the end of 48 hours, hence the BPU-treating period was fixed at 48 hours.

II. Leaf dipping method (IRAC Method No.7): After several preliminary trials, the BPU dipping solutions were diluted at concentration increment of 10-fold. For the susceptible strain, the dilutions were (1) CFA: 1.67, 0.167, 0.0167 and 0.00167ppm, (2) TFB: 5.0, 0.5, 0.05 and 0.005ppm, (3) HXF: 3.33, 0.333, 0.0333 and 0.00333ppm, and (4) FFO: 3.33, 0.333, 0.0333 and 0.00333ppm.

III. In testing the resistant DBM, the BPU solutions were also set at 10-fold increment except HXF, and they are (1) CFA: 500, 50, 5 and 0.5ppm, (2) TFB:1500, 150, 15 and 1.5ppm, (3) HXF: 64, 16, 4 and ppm, and (5) FFO: 1000, 100, 10 and 1ppm.

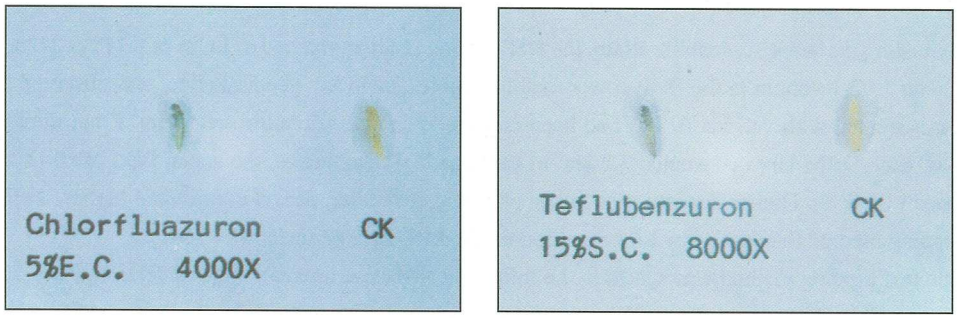
IV. Leaf discs were cut by a stainless steel sampler from cabbage leaves which were washed and air-dried, and the diameter of leaf disc was 3.5 cm. Total of 5 leaf discs were prepared for each dosage and leaf discs were dipped for 5 seconds in BPU solutions containing 333 ppm Triton X-100 then left to air-dry. For control test, cabbage leaf discs were dipped in distilled water with 333 ppm Triton X-100 only and were then air-dried on wire racks. In each petri dish, three leaf discs were used at the beginning, and changed to the other two treated leaf discs at 48-hour interval until pupation. Before using, treated leaf discs were stored in a plastic bag at 5℃. Total of sixty DBM larvae in 4 subsets were subjected to each BPU dosage.

V. The synergist treatment: To measure the synergistic effect of PB on BPUs, mixing ratio of 1: 5 for BPU: PB was used for all the synergistic tests.

VI. Definition of replicates in this study is the same as that defined by Robertson and Preisler[12]: "unless each group of treated with any concentration is treated with a fresh formulation, and (to be absolutely sure) at a different time; it is not a separate replicate" and related discussions.

Response assessment:

The assessments were made at 24, 48, 72, 96 and 120 hours after the start of treatment. Both the dead and the affected (pupae abnormality, Fig.2) were included in the percent of response (mortality). Analysis was made by correcting with control first, and then analyzed by probit[6]. If the mortality in control was over 8 %, the test was abandoned and repeated.



(a) chlorfluazuron (b) teflubenzuron

Fig. 2. Abnormal pupation (left) compared to normal pupation (right) after treated with BPUs

Antibiotic treatment:

Occasionally, the field-collected DBM strains were affected by diseases, which caused high mortality in the propagated population or testing. When this happened, the antibiotic treatment is helpful to reduce the problem. By adding 300ppm tetracycline-HCl in BPU solutions, the disease of DBM can be reduced while causing no interference in the BPU-susceptibility determination.

RESULTS

Based on Celamerck Document No. 13406-522-02, TFB residue analysis method was satisfactory in analyzing three other BPUs i.e., CFA, HXF and FFO. The average weight of cabbage leaf used was 0.0436g/cm^2 and was determined in 6 replicates. Recoveries of the above 4 BPUs from treated cabbage leaves were 67±11.0, 73±7.5, 70±8.6 and 77±5.1 % for TFB, CFA, HXF and FFO, respectively.

The BPU-deposition resulted from dipping was 0.70-1.12μg/cm^2 comparing with that of 0.28-0.33 μg/cm^2 by spraying (Table 1). In other words, the BPU deposition on leaf disc was 3.29-fold higher by dipping than spraying. The BPU deposition study was performed with BPU solutions of 100ppm, and the correlation between applied concentration and the deposited dosage i.e., the deposition factor K, were given in Table l. Hence, the BPU deposition of other concentration can also be estimated.

Table 1. The BPU dose on leaf disc by either spraying or dipping treatment with 100 ppm BPU solution, determined by the HPLC analysis[z]

BPUs	Spraying(X)	Dipping(Y)	X/Y×100 %	K
	(μg/cm^2 leaf±S.E.)			
CFA	0.28±0.025	0.890±0.102	31.5 %	0.890
TFB	0.23±0.012	0.700±0.106	32.9 %	0.700
HXF	0.33±0.023	1.127±0.202	29.2 %	1.127
FFO	0.30±0.023	1.080±0.138	27.8 %	1.080
			Mean = 30.4±2.3 %	

[z] The correlation of the BPU dose on leaf-disc by spray(X) and dipping (Y) :
Y=F(X)=- 0.34594 + 4.5472X
Coeff. of determin.(R^2)= 0.94963
Coeff. of correl.(R)= 0.97449
S.E. of estimation = 0.53913
K=μg/cm^2/ppm

By testing the same susceptible strain, the leaf dipping method resulted in LC_{50}s of 0.119-0.242ppm comparing with 1.02-1.96ppm in the spray tower method (Table 2). No anti-feeding effect was observed since the leaf discs treated with various BPUs had been consumed as that of control treatment. From third instar to pupation, each DBM larva consumed 3.2 cm^2 of cabbage leaf disc, hence, the actual LD_{50} of BPUs could be calculated (Table 3). Higher deposition together with longer feeding period contributed higher sensitivity of leaf dipping method than the spray tower method on the BPU dosage scale.

The leaf dipping method was tested in the following two experiments : first, to determine the sensitivity of the susceptible DBM to 4 assigned BPUs and second, to test the applicability in detecting BPU-resistance in three DBM strains (RS1, RS2 and RS3). For each DBM strain and every assigned BPU, test was conducted in 5 to 7 replicates, usually in two generations. This is not a particular concern in the susceptible strain, but the

decline of BPU-resistance in the field-collected strains may cause problem. In two consecutive generations, the decline of BPU-resistance is minimum[3], hence the results were analyzed together. The original LC_{50}s, their 95 % fiducial limits and dosage response slopes of all 4 DBM strains were listed in appendixes 1-4. The LC_{50} data were collectively summarized (Table 4) for discussion.

Table 2. Susceptibilities of the suseptible DBM to 4 assigned BPUs determined by two different treating methods

BPUs	LC_{50} in ppm, Mean±S.D	
	Leaf dipping method	Spray tower method
CFA	0.119±0.108	1.96±1.86
TFB	0.129±0.072	1.05±0.81
HXF	0.242±0.213	1.02±0.56
FFO	0.234±0.188	1.21±1.04

Table 3. Estimated LD_{50}s of 4 BPUs in the susceptible and 3 resistant strains of DBM

Strains	LD_{50}(ng/larva)[z]			
	CFA	TFB	HXF	FFO
Susceptible	3.39	2.89	8.72	8.09
RS1	159.99	933.69	143.48	521.64
RS2	114.15	222.34	207.44	393.81
RS3	1,315.21	1,903.20	392.94	6,643.08

[z]Calculated on base of 3.207 cm^2/larvae, feeding period was from 3rd instar to pupation.

Table 4. The summarized analysis of LC_{50}s of the susceptible and 3 resistant DBM strains

Strains	LC_{50} in ppm, Mean ±S.D.			
	CFA	TFB	HXF	FFO
Susceptible strain	0.119±0.108	0.129±0.072	0.242±0.213	0.234±0.188
RS1	5.62±3.24	41.70±29.30	3.98±1.25	15.10±4.87
RS2	4.01±4.89	9.93±6.67	5.74±4.59	11.40±12.60
RS3	46.20±48.70	85.00±80.30	10.90±6.05	192.30±222.60

The LC_{50}s of susceptible DBM to 4 assigned BPUs were measured at 0.119, 0.129, 0.242 and 0.234 ppm for CFA, TFB, HXF and FFO, respectively (Table 5). The distribution of 4 BPUs' susceptibilities and their standard deviation in the susceptible strain were close to a factor of 2 in both 95 % and 99 % upper fiducial limits. When multiply an agreed constant, it is possible to set a detection threshold in leaf dipping method for monitoring the BPU-resistance in DBM.

The reproducibility of leaf dipping method was examined by testing the same susceptible strain at 6 generations later, and the re-determined LC_{50} of 4 BPUs (Table 6) all fit into the original fiducial limits (Table 5), hence, the result of first experiment is satisfactory. Several previously reported LC_{50}s of BPUs in susceptible DBM strains (Table 7) were also comparable to the results of this study.

In the second experiment, LC_{50}s of 3 resistant strains (RS1, RS2 and RS3) were determined at 3.98 to 192.3 ppm for the four BPUs (Table 4), which were significantly higher than those of susceptible strain. The Student's t-distribution test was then used to justify the separation from the susceptible strain (Table 8). Except the RS2/chlorfluazuron test was on the edge of 95 % confidence, all other comparison indicated that leaf dipping method can positively verify BPU-resistance in resistant strains.

Table 5. Mean and fiducial limits of BPU susceptibilities in the susceptible DBM in the n generation

BPUs	BPU susceptibility (LC_{50}, in ppm)	
	Mean	95 % Fiducial limits
CFA	0.1193	0.01971～0.21887
TFB	0.1294	0.06250～0.19636
HXF	0.2421	0.04487～0.43941
FFO	0.2339	0.06041～0.40731

Table 6. Mean and fiducial limits of BPU susceptibilities determined in the n+6 generation of the susceptible DBM

BPUs	BPU susceptibility (LC_{50}, in ppm)	
	Mean	95 % Fiducial limits
CFA	0.103	0.0757～0.1388
TFB	0.177	0.1313～0.2372
HXF	0.211	0.0545～0.5689
FFO	0.125	0.0789～0.1888

Table 7. Comparison of BPU susceptibilities of DBM in published literatures

Strains	BUP susceptibilities, LC_{50} in ppm				References
	TFB	CFA	HXF	FFO	
Susceptible					
Perng-FS	0.068	0.12	—	—	Perng *et al.*(1988)
Syed-suscept.	0.46	0.057	—	—	Syed(1992)
Kobayashi-suscept.	—	0.1	0.15	—	Kobayashi *et al.*(1992)
Fauziah-FS	0.018	0.015	0.043	0.008	Fauziah & Wright(1991)
Field susceptible					
Perng-MD	0.14	0.15	—	—	Perng *et al.*(1988)
Kobayashi-BBT	—	13.0	—	—	Kobayashi *et al.*(1992)
-BK	—	1.4	—	—	
Fahmy-TL	0.74	0.99	—	—	Fahmy *et al.*(1991)
-BK	0.41	1.03	—	—	
Fahmy-TL	—	1.02	—	—	Fahmy and Miyata(1992)
-BK	—	0.57	—	—	
TARI-susceptible					
-IL	0.1294	0.1193	0.2421	0.2339	(Table 6)

Table 8. Student's t-distribution test (independent) of LC_{50} between the susceptible and 3 resistant DBM strains

Resistant strain	Susceptible strain			
	CFA	TFB	HXF	FFO
RS1	RS1 vs.S t = 4.571 df = 10 p = 0.00102	RS1 vs.S t = 3.785 df = 11 p = 0.00302	RS1 vs.S t = 7.869 df = 10 p = 0.00002	RS1 vs.S t = 8.210 df = 10 p = 0.00000
RS2	RS2 vs.S t = 2.106 df = 12 p = 0.05700	RS2 vs.S t = 3.886 df = 12 p = 0.00216	RS2 vs.S t = 3.167 df = 12 p = 0.00812	RS2 vs.S t = 2.368 df = 11 p = 0.03730
RS3	RS3 vs.S t = 2.520 df = 11 p = 0.02840	RS3 vs.S t = 2.815 df = 11 p = 0.01680	RS3 vs.S t = 4.703 df = 11 p = 0.00064	RS3 vs.S t = 2.330 df = 10 p = 0.04204

The LC_{50}s of the susceptible DBM strain of this study can serve as a reference for other monitoring program with no susceptible strain as its standard from the following observations: (1) the LC_{50}s (Table 5) are comparable to other susceptible DBMs (Table 7), (2) the IL-strain which was collected before the BPUs registration in Taiwan had proven with no BPU-resistance, and (3) if the monitored DBM had a susceptibility similar to that of the IL-strain, BPU should be effective in the field.

From the BPU-resistance information gathered in 3 field-collected DBM strains, HXF suffered the least BPU-resistance. By a closer examination of b-value (slope) in probit analysis (Table 9 and Appendix 5), higher b-value was observed in HXF. The significance of this slope shift was confirmed in the LSD test (Table 10). Higher b-value of HXF in the resistant DBM strains indicated smaller resistance ratio at the higher mortality

range, for example the LC_{90}. It is meaningful in the field practice since farmers only concern about the mortality level above 90 %. Nevertheless, HXF did meet resistance in DBM as other BPUs.

Table 9. The summarized analysis of b-value (slope) of the susceptible and 3 resistant DBM strains

Strains	b-value, Mean±S.D.			
	CFA	TFB	HXF	FFO
Susceptible strain	0.836±0.48	0.861±0.32	0.994±0.62	0.971±0.37
RS1	0.850±0.19	0.540±0.10	1.426±0.24	0.576±0.13
RS2	0.650±0.18	0.645±0.14	1.398±0.26	0.614±0.12
RS3	0.715±0.25	0.465±0.05	1.383±0.34	0.482±0.03

Table 10. The LSD analysis of b-value of BPU susceptibilities of the susceptible and 3 resistant DBM strains

Strains	Least Significant Difference			
	Statistical parameters		b-value difference	
Susceptible strain	F-ratio=0.1967 F-prob.=0.8976		no difference in b-values	
RS1	Error mean square=0.0310	Mean	Sample size	Separation
	df=17, Alpha level=0.05	0.540(FFO)	6	a
	Harmonic average sample size=5.22	0.576(TFB)	5	a
	Table value from Student's t=2.11	0.850(CFA)	5	b
	LSD value=0.2299	1.426(HXF)	5	c
RS2	Error mean square=0.0355	Mean	Sample size	Separation
	df=23, Alpha level=0.05	0.614(FFO)	6	a
	Harmonic average sample size=6.72	0.645(TFB)	7	a
	Table value from Student's t=2.069	0.650(CFA)	7	a
	LSD value=0.2126	1.398(HXF)	7	b
RS3	Error mean square=0.0499	Mean	Sample size	Separation
	df=19, Alpha level=0.05	0.465(TFB)	6	a
	Harmonic average sample size=5.71	0.482(FFO)	5	a
	Table value from Student's t=2.093	0.715(CFA)	6	a
	LSD value=0.2765	1.383(HXF)	6	b

No conclusive result can be reached in the piperonyl butoxide study by the leaf dipping method. Since PB treatment in most studies was conducted by treating insect topically rather than feeding, the leaf dipping method used here only treating insects through feeding may not be suitable for the PB synergistic study.

DISCUSSIONS AND CONCLUSION

Proposed leaf dipping method had been proven with higher sensitivity compared to the spray tower method, which was due to the higher BPU deposition and the longer treating period. Dipping method had been used by many BPU-resistance investigations[4,5,9,13] and the results were summarized for comparison (Table 7). In the susceptible strains, only Fauziah and Wright[5] obtained 5.6 to 29-fold higher sensitivity than other studies, but their experiment was conducted at 20°C (lower than others) by using the second instar larvae (instead of the third instar used by others), and the observation period was 17 days (longer than the 5-day period of others), which was significantly different from other studies. Similar phenomenon was observed when testing BPUs on Colorado beetle, *Leptinotarsa decemlineata*, the sensitivity to IGRs mainly depends on the period of time after treatment, and higher activity was found on young larvae[10]. Perng *et al.*[11] and Kobayashi *et al.*[9] conducted experiments at 25°C with a 5-day observation after treatment, which were similar to the IRAC Method No. 7.

Field-collected susceptible strains in previous reports (Table 7) showed slightly higher LC_{50}s than the susceptible strain except Perng *et al.*[11] measured the Taiwan MD-native strain, which had almost the same susceptibility as the laboratory susceptible FS-strain. TARI tested a Taiwan native suseptible IL-strain and demonstrated the same susceptibility as that of MD-strain.

In conclusion, by adopting the leaf dipping method (IRAC Method No.7), the BPU sensitivities of the susceptible IL-strain DBM were equivalent or similar to the results of Perng *et al.*[11], Syed[13] and Kobayashi *et al.*[9], and also more sensitive than the field DBM strains of Kobayashi *et al.*[9] and Famhy and Miyata[4].

By adopting the IRAC Method No.7 in testing the field-collected BPU resistant strains (RS1-3), the LC_{50}s were statistically higher than that of susceptible IL-strain, and the BPU-resistance had been confirmed in those DBM strains.

During the experiment, no anti-feeding or dosage related feeding retardation was observed for BPU. Fisk and Wright[7] also reported no repellent action in testing acylurea on *Spodoptera exempta.*

Several BPU resistance studies claimed that the resistance stemmed from mixed function oxidases (MFO)[3,5,11]. Nevertheless, by mixing PB into BPU dipping solution resulted in inconsistent synergistic effect in this investigation. Since all previous studies conducted the PB treatment by either spraying or topical application rather than feeding, we doubted that through feeding only, the tratment may not be appropriate for measuring the synergistic action of PB.

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APPENDIX 1 : The BPU-susceptibilities of susceptible DBM

BPUs	LC_{50} in ppm	95 % Fiducial limit	Slope±S.D.
CFA			
rep.1	0.040	0.021~0.079	0.613±0.088
rep.2	0.098	0.053~0.180	0.685±0.092
rep.3	0.273	0.153~0.485	0.822±0.103
rep.4	0.021	0.008~0.052	0.451±0.083
rep.5	0.256	0.187~0.351	1.910±0.234
rep.6	0.131	0.076~0.226	0.784±0.092
rep.7	0.016	0.008~0.032	0.590±0.084
TFB			
rep.1	0.139	0.090~0.214	1.063±0.110
rep.2	0.231	0.090~0.590	0.410±0.078
rep.3	0.053	0.030~0.093	0.753±0.092
rep.4	0.034	0.015~0.080	0.537±0.084
rep.5	0.180	0.099~0.326	0.852±0.135
rep.6	0.176	0.121~0.255	1.331±0.139
rep.7	0.093	0.061~0.143	1.082±0.113
HXF			
rep.1	0.180	0.097~0.332	0.665±0.085
rep.2	0.264	0.159~0.436	0.880±0.101
rep.3	0.035	0.017~0.075	0.575±0.085
rep.4	0.123	0.064~0.237	0.627±0.086
rep.5	0.374	0.252~0.556	1.292±0.158
rep.6	0.648	0.484~0.868	2.292±0.300
rep.7	0.071	0.038~0.135	0.627±0.084
FFO			
rep.1	0.338	0.214~0.535	1.034±0.115
rep.2	0.231	0.131~0.406	0.764±0.093
rep.3	0.076	0.034~0.168	0.498±0.081
rep.4	0.589	0.321~1.081	0.772±0.098
rep.5	0.096	0.064~0.145	1.383±0.188
rep.6	0.061	0.036~0.103	0.797±0.092
rep.7	0.246	0.171~0.356	1.549±0.187

APPENDIX 2： The BPU-susceptibilities of resistant DBM strain 1 (RS1)

BPUs	LC_{50} in ppm	95 % Fiducial limit	Slope±S.D.
CFA			
rep.1	3.0	1.6～ 5.6	0.766±0.097
rep.2	2.5	1.4～ 4.5	0.776±0.098
rep.3	4.8	2.5～ 9.4	0.641±0.087
rep.4	7.6	4.7～ 12.3	0.906±0.100
rep.5	10.2	6.7～ 15.3	1.160±0.120
TFB			
rep.1	11.7	3.9～ 34.9	0.388±0.078
rep.2	52.9	25.7～108.8	0.534±0.078
rep.3	92.7	52.0～165.0	0.718±0.087
rep.4	45.2	22.2～ 92.0	0.546±0.080
rep.5	21.5	9.7～ 47.5	0.506±0.080
rep.6	26.2	12.6～ 54.7	0.545±0.081
HXF			
rep.1	2.1	1.3～ 3.5	1.096±0.162
rep.2	4.5	3.2～ 6.5	1.492±0.182
rep.3	4.2	3.0～ 5.8	1.763±0.220
rep.4	5.5	3.8～ 7.9	1.453±0.177
rep.5	3.6	2.4～ 5.3	1.324±0.173
FFO			
rep.1	7.6	2.8～ 20.4	0.400±0.097
rep.2	16.3	9.0～ 29.6	0.652±0.107
rep.3	20.5	10.9～ 38.4	0.606±0.106
rep.4	13.5	5.9～ 31.1	0.475±0.078
rep.5	17.5	10.0～ 30.6	0.747±0.090

APPENDIX 3 : The BPU-susceptibilities of resistant DBM strain 2 (RS2)

BPUs	LC_{50} in ppm	95 % Fiducial limit	Slope±S.D.
CFA			
rep.1	0.2	0.1～ 1.2	0.345±0.058
rep.2	1.7	1.4～ 5.2	0.709±0.086
rep.3	1.1	0.4～ 3.2	0.516±0.081
rep.4	0.1	0.0～ 0.5	0.581±0.159
rep.5	3.7	2.2～ 6.2	0.892±0.104
rep.6	13.9	7.0～27.6	0.717±0.097
rep.7	6.4	3.6～11.3	0.789±0.096
TFB			
rep.1	0.6	0.1～ 3.1	0.486±0.086
rep.2	3.4	1.1～10.3	0.493±0.080
rep.3	6.4	2.7～15.6	0.534±0.079
rep.4	18.3	9.6～34.9	0.649±0.086
rep.5	10.6	5.9～19.0	0.778±0.096
rep.6	14.4	8.1～25.6	0.731±0.090
rep.7	15.8	9.4～26.3	0.834±0.096
HXF			
rep.1	1.3	0.9～ 1.9	1.349±0.178
rep.2	3.1	2.2～ 4.5	1.131±0.133
rep.3	1.8	1.2～ 2.8	1.045±0.138
rep.4	2.6	1.9～ 3.6	1.816±0.245
rep.5	12.5	9.2～17.0	1.438±0.269
rep.6	8.7	6.5～11.6	1.422±0.174
rep.7	10.2	7.8～13.3	1.586±0.187
FFO			
rep.1	3.8	1.7～ 8.2	0.626±0.083
rep.2	2.4	0.8～ 7.1	0.477±0.077
rep.3	0.8	0.2～ 3.1	0.495±0.083
rep.4	8.1	4.0～16.2	0.626±0.085
rep.5	32.3	14.4～72.5	0.618±0.090
rep.6	21.2	12.1～37.2	0.839±0.103

APPENDIX 4： The BPU-susceptibilities of resistant DBM strain 3 (RS3)

BPUs	LC_{50} in ppm	95 % Fiducial limit	Slope±S.D.
CFA			
rep.1	104.4	66.8～163.3	0.915±0.112
rep.2	112.3	38.6～326.3	0.374±0.074
rep.3	24.5	12.2～ 49.3	0.491±0.076
rep.4	3.4	2.2～ 5.4	1.040±0.119
rep.5	15.7	8.9～ 27.6	0.706±0.086
rep.6	16.8	9.8～ 28.9	0.766±0.090
TFB			
rep.1	136.0	67.8～272.8	0.510±0.078
rep.2	112.5	43.9～287.7	0.364±0.074
rep.3	208.5	89.4～485.9	0.435±0.076
rep.4	17.9	8.1～ 39.7	0.514±0.081
rep.5	15.6	6.4～ 38.0	0.460±0.079
rep.6	19.2	8.6～ 42.7	0.504±0.080
HXF			
rep.1	19.0	14.2～ 25.3	1.460±0.153
rep.2	17.1	12.4～ 23.5	1.244±0.134
rep.3	12.0	7.6～ 19.0	0.789±0.114
rep.4	5.1	3.5～ 7.4	1.439±0.176
rep.5	5.8	4.1～ 8.3	1.526±0.183
rep.6	6.6	4.8～ 9.1	1.840±0.219
FFO			
rep.1	196.2	81.6～471.5	0.445±0.078
rep.2	576.0	210.9～1573.5	0.478±0.082
rep.3	88.8	40.7～193.8	0.453±0.077
rep.4	48.3	22.7～103.0	0.502±0.061
rep.5	52.4	25.4～108.1	0.531±0.062

Appendix 5 : The probit slopes (b-value) of the susceptible and 3 resistant DBM strains to BPUs

Strains	b-value			
	CFA	TFB	HXF	FFO
Susceptible strain	0.613	1.063	0.665	1.034
	0.685	0.410	0.880	0.764
	0.822	0.753	0.575	0.498
	0.451	0.537	0.627	0.772
	1.910	0.852	1.292	1.383
	0.784	1.331	2.292	0.797
	0.590	1.082	0.627	1.549
RS1	0.766	0.388	1.096	0.400
	0.776	0.534	1.492	0.652
	0.641	0.718	1.763	0.606
	0.906	0.546	1.453	0.475
	1.160	0.506	1.324	0.747
	—	0.545	—	—
RS2	0.345	0.486	1.349	0.626
	0.709	0.493	1.131	0.477
	0.516	0.543	1.045	0.495
	0.581	0.649	1.816	0.626
	0.892	0.778	1.438	0.618
	0.717	0.731	1.422	0.839
	0.789	0.834	1.586	—
RS3	0.915	0.510	1.460	0.445
	0.374	0.364	1.244	0.478
	0.491	0.435	1.789	0.453
	1.040	0.514	1.439	0.502
	0.706	0.460	1.526	0.531
	0.766	0.504	1.840	—

應用 IRAC Method No. 7 (葉片浸藥法)偵測小菜蛾對 BPU 類生長調節劑抗性之研究[1]

鄭 允 高靜華[2]

摘要：本研究係比較 IRAC（Insecticide Resistance Action Committee）之建議方法，IRAC Method No. 7（葉片浸藥法），與噴霧塔兩種方法測試小菜蛾對 BPU 類生長調節劑抗藥性之差異。葉片之沾藥量方面，葉片浸藥法高出噴霧塔法 3.29 倍。葉片浸藥法之測試時間爲三齡蟲至化蛹，較一般噴霧塔方法之 48 小時爲長，未發現小菜蛾對 BPU 類生長調節劑處理之葉片有拒食現象。由於葉片浸藥法採用較高的處理濃度與較長的測試時間，所得到之感度也比噴霧塔法更高。

以葉片浸藥法測試感性與抗性品系小菜蛾對 chlorfluazuron、teflubenzuron、hexaflumuron 及 flufenoxuron 四種 BPU 類生長調節劑感度，並以感性品系測試間隔六代之 LC_{50} 差異，以確認此一方法之重現性。經以 Independent Student's t-test 分析，證實葉片浸藥法適用於小菜蛾對 BPU 類生長調節劑抗藥性之檢測，感性品系小菜蛾與供試之 3 個抗性品系小菜蛾 LC_{50} 在 95 %之信賴區間有顯著差異，因而可檢出抗性。

四種供試之 BPU 類生長調節劑中，除 hexaflumuron 須以 4～5 倍之濃度間隔測試外，餘均以 4 至 5 個連續稀釋十倍之藥劑濃度測試，葉片浸藥法可有效檢測小菜蛾對 BPU 類生長調節劑之抗藥性。因 IRAC Method No.7 係以餵食處理，不適用於 BPU 類生長調節添加協力劑 piperonyl butoxide（PB）之測試。

關鍵詞：小菜蛾、BPU 類生長調節劑、抗藥性、IRAC Method No. 7、葉片浸藥法。

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